(12) 按照专利合作条约所公布的国际申请

(19) 世界知识产权组织
国 际 局

(43) 国际公布日:
2003年10月9日(09.10.2003)

PCT

(10) 国际公布号:
WO 03/082838 A1

(51) 国际分类号[7]: C07D 277/22, 277/38, 263/30, 263/48, 207/30, 207/34, 401/12, 403/12, 405/12, 407/12, 417/12, A61K 31/40, 31/41

(21) 国际申请号: PCT/CN03/00213

(22) 国际申请日: 2003年3月25日(25.03.2003)

(25) 申请语言: 中文

(26) 公布语言: 中文

(30) 优先权:
02111230.4 2002年4月2日(02.04.2002) CN

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(81) 指定国(国家): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW

(84) 指定国(地区): ARIPO专利(GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), 欧亚专利(AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), 欧洲专利(AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI专利(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

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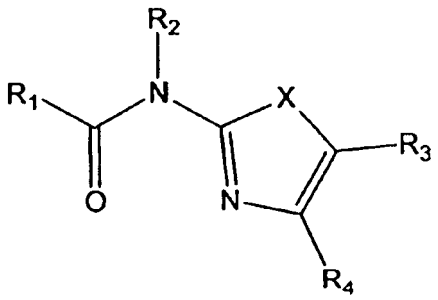
(54) Title: NEW METHIONINE AMINOPEPTIDASE INHIBITOR

(54) 发明名称: 一类甲硫氨酰氨肽酶抑制剂



(I)

(57) Abstract: The invention provides a new methionine aminopeptidase inhibitors with the following formula (I): wherein R_1, R_2, R_3, R_4, R_5, R_6, and X have the meanings given in the description. Pharmacological experiment shows that they display good inhibition activity for methionine aminopetptidase.

[见续页]

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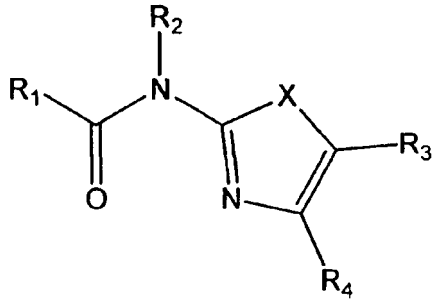
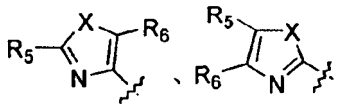
(57) 摘要

本发明提供了一类新的甲硫氨酰氨肽酶抑制剂，该类抑制剂结构如下：



其中 R_1 为 C_1-C_4 的烷基、取代烷基、C_3-C_6 的环烷基、取代环烷基、芳基、吡啶基；由 C_1-C_4 的烷基、硝基、羧基、醛基、烷氧基、胺基、酰 氨基、巯基的取代芳基、取代吡啶基和具有如下结构的杂环或取代杂环



R_5、R_6 为 H、C_1-C_4 的烷基、取代烷基、C_3-C_6 的环烷基、取代环烷基、芳基、吡啶基、硝基、羧基、醛基、烷氧基、胺基、酰氨基、巯基的取代芳基、取代吡啶基；

R_2 为 H、C_1-C_4 烷基、取代烷基、芳基、由 C_1-C_4 的烷基硝基、羧基、醛基、烷氧基、胺基、酰 氨基、巯基的取代芳基；

R_3 为 H、C_1-C_4 烷基、取代 C_1-C_4 烷基、卤素；芳基、取代芳基；

R_4 为 H、C_1-C_4 烷基、取代烷基、取代芳基；

X 为 O、S、N、杂原子。

经药理试验证明对甲硫氨酰氨肽酶具有较好的抑制活性。

一类甲硫氨酰氨肽酶抑制剂

技术领域

本发明涉及一类小分子有机化合物对甲硫氨酰氨肽酶（MetAPs）显示了高的抑制活性，并对不同的 MetAPs 亚型表现出一定的选择性，因而可作为一类新的抗肿瘤和抗菌药物研究的先导化合物。

背景技术

在原核细胞的细胞质内所有蛋白质的翻译都起始于 N 端的甲硫氨酸，然而在真核细胞、线粒体和叶绿体中蛋白质的翻译起始于 N—甲酰化甲硫氨酸，甲酰化基团一般是在伴随翻译的过程中被去甲酰化酶作用后除去。不论在真核细胞还是在原核细胞中，甲硫氨酰氨肽酶（MetAPs）有选择性的切除新生蛋白或多肽链的 N 端甲硫氨酸。新生蛋白或多肽链 N 端甲硫氨酸的切除对于蛋白的翻译后修饰以及在细胞的准确定位和功能的正常发挥起着关键的作用。

大肠杆菌甲硫氨酰氨肽酶是最先发现的甲硫氨酰氨肽酶成员，随后人们分别在酵母和哺乳动物中也发现了结构和功能类似的酶。从序列的比较来看，这类甲硫氨酰氨肽酶的 C 端有着很高的同源性，但是 N 端却延伸了一段类似锌指结构的序列，这段序列结构后来被实验证实是与核糖体结合序列，对蛋白酶活性没有影响(J. Biol. Chem. 1990; 265: 19892-19897)。该类酶与大肠杆菌甲硫氨酰氨肽酶被划分为 I 型酶；后来人们从猪肝中分离纯化并克隆到另一类甲硫氨酰氨肽酶(J, Biol. Chem. 1992;267:20667-20673)，序列比较其 C 端与大肠杆菌甲硫氨酰氨肽酶的同源性要比 I 型酶低一些（C 末端插入了一段约 60 个氨基酸的序列），在其 N 端是由一些多聚中性和酸性氨基酸残基取代锌指结构序列（这部分结构被证实是真核细胞内蛋白翻译起始因子磷酸化的抑制子，并不影响蛋白酶的活性），为了与 I 型区分，人们将该类酶分为 II 型酶，如陆续发现的酵母、果蝇、小鼠和人的 II 型甲硫氨酰氨肽酶。

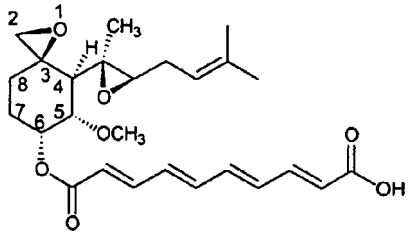
甲硫氨酰氨肽酶广泛存在于生物界各种生物的细胞中，对底物具有一定的选择性。不论是内源性的蛋白还是体外重组表达的蛋白或是多肽作为底物的实验都表明(Biochemistry. 1987;26:8242-8246)，只有当蛋白或多肽底物 N 端甲硫氨酸后的第二个氨基酸（P_1'）属于非极性链小于 3.68Å，才能被甲硫氨酰氨肽酶酶解. 目前认为 P_1' 位置的氨基酸是以下七种氨基酸中任一便能被甲硫氨酰氨肽酶作用：Gly、Ala、Ser、Thr、Pro、Val 和 Cys (J. Bacteriol. 1987;169:751-757;Biochemistry. 1999;38:14810-14819).

甲硫氨酰氨肽酶在细胞内的基本功能是切除细胞内新合成蛋白的N端甲硫氨酸，为蛋白质的后续功能奠定基础.新生蛋白或多肽链 N 端甲硫氨酸的切除，对于蛋白的翻译后修饰、在细胞的准确定位、蛋白的直接降解(J.Biol.Chem.1982;257:3532-3536)和功能的正常发挥起着关键的作用。

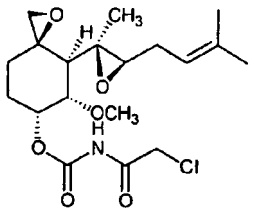
目前甲硫氨酰氨肽酶的抑制剂根据作用方式，我们可将它们分为共价结合抑制剂和非共价结合抑制剂。另外还有一类抑制剂是过度态类似物和反应产物，它们也具有相似于Bestatin类似物的抑制机理(Biochemistry.1999;38:14810-14819)。

通过共价结合方式抑制甲硫氨酰氨肽酶的化合物是一类能够特异抑制血管内皮细胞生长的药物，烟曲霉素（fumagillin）和它的衍生物TNP-470（IC_{50}为纳摩尔级）。研究表明，人源的 hMetAP II 是细胞内 fumagillin 和 TNP-470 的作用靶点，该类化合通过共价修饰 hMetAP II 催化区保守氨基酸残基 His，从而达到抑制该蛋白酶的活性(Proc.Natl.Acad.Sci.USA.1998;95:15183-15188)。

非共价抑制剂是针对于 MetAPs 的底物特性，以亮氨酰氨肽酶的有效抑制剂 Bestatin 为模板改造的底物类似物抑制剂。将 Bestatin 的 P_1和 P_1'位置替之以异亮氨酸和丙氨酸，结果是一个不能被水解的底物类似物，它的抑制活性 IC_{50} 为 5μM，是目前报道的 eMetAP 最好的抑制剂。

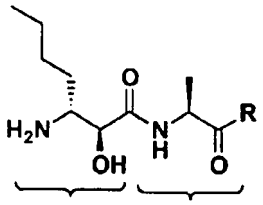


fumagillin



TNP-470

MetAPs 的几个共价结合抑制剂



AHHpA P_1 Ala P_1'

R= Leu-Val-Phe-Ome

eMetAP 的非共价抑制剂

近几年来，肿瘤化疗取得了相当的进步，肿瘤患者生存时间明显延长，特别是对白血病、恶性淋巴瘤等的治疗有了突破，但对危害人类生命健康最严重的、占恶性肿瘤 90%

以上的实体瘤的治疗未能达到满意的效果。药学家和肿瘤 学家越来越深刻地认识到：要提高肿瘤治疗的疗效，必须从肿瘤发生发展的机制着手，才能取得新的突破性进展。抗肿瘤药物正从传统的细胞毒性药物，向针对机制的多环节作用的新型抗肿瘤药物发展。肿瘤的血管系统是一个崭新的、有希望的抗肿瘤治疗靶点，因为肿瘤的生长和转移依赖于新生血管生成（angiogenesis），肿瘤既可通过肿瘤血管从宿主获取营养和氧气，又可通过肿瘤血管源源不断地向宿主输送转移细胞，并在机体的其它部位继续生长和诱导血管生成，导致肿瘤转移。通过抑制肿瘤血管生成来抑制肿瘤血管生成是当今抗肿瘤药物研究最活跃的领域之一。烟曲霉素及其类似物所具有的抗肿瘤作用，正是通过作用于肿瘤的血管系统而实现，通过特异性的抑制血管内皮细胞生长进而抑制肿瘤生长。这说明，甲硫氨酰氨肽酶特别是 hMetAPII 可作为一种新型的抗新生血管生成药物的作用靶点，对它有效而特异性的抑制剂可作为新型的抗肿瘤药物。

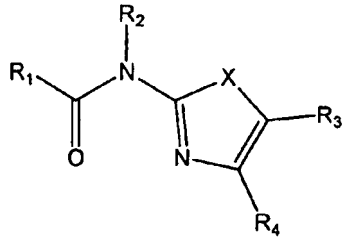
在原核生物中仅有 $MetAP_1$, MetAPs 基因的敲除实验表明，大肠杆菌、伤寒沙门氏菌以及酵母都不能再继续生长，这说明 $MetAP_1$ 在原核生物的生长过程中非常重要的作用，因而能选择性抑制 $MetAP_1$ 的化合物有可能作为一类新型的抗细菌感染药物。

发明内容

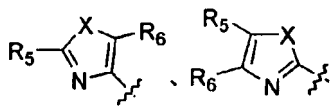
发明目的：本发明设计与合成新型的小分子有机化合物作为 MetAPs 抑制剂，并对其结构与活性关系进行深入的研究，在阐明 MetAPs 在病理条件下的作用机制的同时找到抗癌、抗感染药物的先导化合物。

本发明的另一个目的是提供该类化合物的合成方法。

本发明所述一类甲硫氨酰氨肽酶抑制剂具有如下结构：



其中 R_1 为 C_1-C_4 的烷基、取代烷基、C_3-C_6 的环烷基、取代环烷基、芳基、吡啶基；由 C_1-C_4 的烷基、硝基、羧基、醛基、烷氧基、胺基、酰 氨基、巯基的取代芳基、取代吡啶基和具有如下结构的杂环或取代杂环



R_5、R_6为 H、C_1-C_4的烷基、取代烷基、C_3-C_6的环烷基、取代环烷基、芳基、吡啶基、硝基、羧基、醛基、烷氧基、胺基、酰氨基、巯基的取代芳基、取代吡啶基；

R_2为 H、C_1-C_4烷基、取代烷基、芳基、由C_1-C_4的烷基、硝基、羧基、醛基、烷氧基、胺基、酰 氨基、巯基的取代芳基；

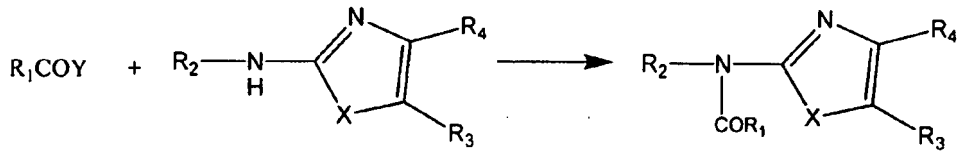
R_3为 H、C_1-C_4烷基、取代C_1-C_4烷基、卤素；芳基、取代芳基；

R_4为 H、C_1-C_4烷基、取代烷基、取代芳基；

X 为 O、S、N、杂原子。

本发明通过下列步骤实施：

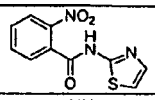
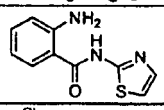
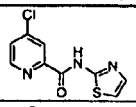
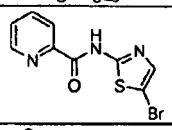
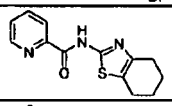
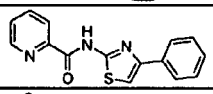
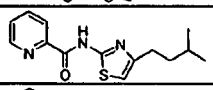
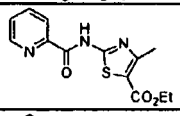
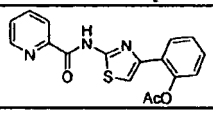
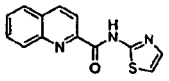
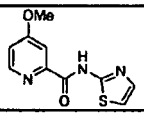
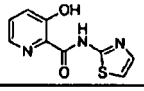
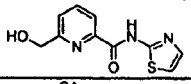
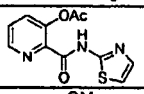
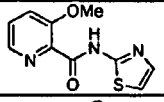
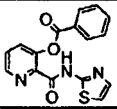
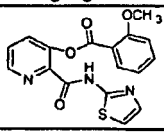
根据化学反应式

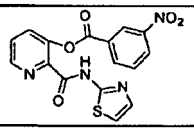
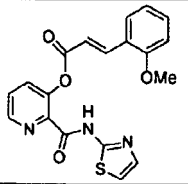
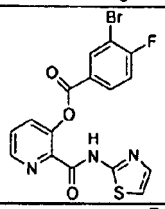
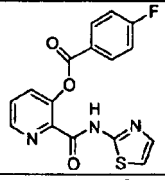
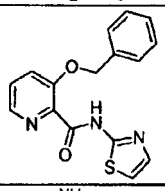
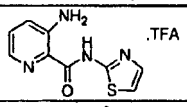
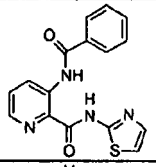
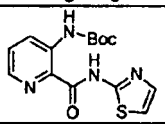
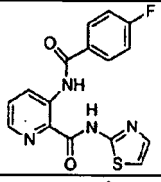
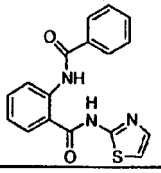
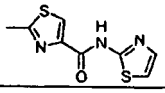


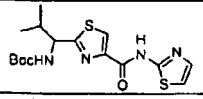
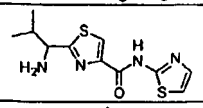
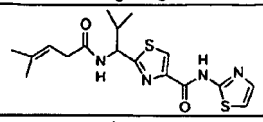
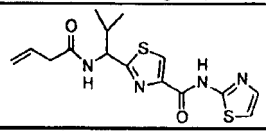
I II III

化合物 I 与 II 缩合得化合物 III，其中 Y 为 OH、Cl 或其它活性基团，化合物 I 与 II 在如下溶剂中进行缩合反应，CH_2Cl_2、DMF、CH_2ClCH_2Cl、甲苯、苯、水、二氧六环或在需要时使用混合溶剂，例如：CH_2Cl_2/DMF（1：1 V/V），所使用的缩合剂根据化合物性质可为 DCC、ECD、DIC、HBTU 等，根据反应需要时加入少量活化剂，例如 HOBT、五氟苯酚、分子筛等，有时反应还需加入碱作催化剂，如三乙胺、二乙丙基乙基胺、吡啶、DMAP、通常反应温度从-20℃—室温，但在某些情况下，则需加热，一般从 50°-130℃，反应时间同样视反应物的活化基团而定，例如 Y 为 Cl 时可在几分钟内完成反应，一些反应则需时间长一些，通常用 TLC 来测定反应完成程度，反应完毕后一般用醋酸乙酯或二氯甲烷、氯仿等溶剂提取，依次用 5%HCl、水、饱和食盐水洗，经干燥后，低温减压除去溶剂，浓缩物经柱层析得最终产物 III，产率视反应物 I 和 II 的性质而变化，从 20%—95%，得到的产物用 NMR 等方法来证明

化合物 II 可根据 J.Org.Chem. 63,196-200(1998)的方法合成。

	化学结构	$IC_{50}(\mu M)$	
		EMetAP	hMetAP1
6		NA	–
7		30% inhibition at 20μg/ml	–
16		4.84	–
17		6. 0	–
18		NA	–
22		NA	–
23		NA	–
24		NA	–
25		NA	–
27		51.0	–
30		9.22	–
31		2.33	–
32		5.82	–
34		8.20	29.0
35		4.49	20.9
36		1.10	28.7
37		1.90	15.4

41		1.91	7.3
44		1.80	–
45		1.35	17.4
46		1.31	7.0
47		5.31	7.8
50		5.56	11.0
51		0.14	–
52		1.25	–
53		0.05	–
54		NA	–
55		0.4	

56		NA	–
57		NA	–
58		NA	–
59		NA	–

NA: no inhibition at 20 μg/mL, "_" no test

生物活性测试

用大肠杆菌系统克隆并大量表达 eMetAP 蛋白，经饱和(NH4)2SO4 沉淀及 Q-Sepharose 柱层析纯化后，得到脱辅基酶(apo-eMetAP)，最后经与适当浓度的两价钴离子孵育后，得到高活性的酶可以进行该酶抑制剂的筛选。

药物筛选模型的测试原理

eMetAP 可以水解合成底物 Met-S-C-Phe 的硫酯键，产物 Met-SH 迅速与过量的 DTNB 反应，产生的 3-羧基-4-硝基硫代苯酚盐在 412 nm 处有吸收（ε 412= 13600 M-1. cm-1）。通过 SpectraMAX 340 检测 412 nm 处的的光吸收变化来确定酶活性。

操作步骤：

采用常规筛选方法（Anal Biochem. **2000**, 280:159-65）

筛选选取 2 μg/mL、20 μg/mL 和 100 μg/mL 三个化合物浓度进行初步筛选，当抑制活性高于 50%时，取 8 个浓度测定该活性化合物 IC_{50}。

本发明的优点：

本发明说明合成的一系列化合物为一类全新结构的甲硫氨酰氨酞酶抑制剂，与现在已知的这类酶的抑制剂相比，结构相对简单，易于制备。而且其中的某些化合物对甲硫氨酰氨酞酶 eMetAP 的抑制活性为当前最好的。

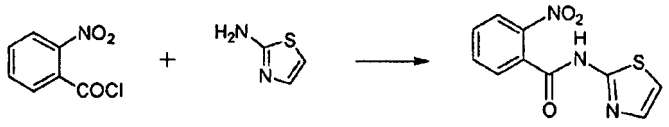
具体实施方式

下面结合具体实施例对本发明作进一步阐述，但不限制本发明。

^{1}H-NMR 用 Varian MercuryAMX300 型仪测定；MS 用 VG ZAB-HS 或 VG-7070 型仪测定，除注明外均为 EI 源（70ev）;所有溶剂在使用前均经过重新蒸馏，所使用的无水溶剂均是按标准方法干燥处理获得；除说明外，所有反应均是在 Ar 气保护下进行并用 TLC

跟踪，后处理时均经饱和食盐水洗和无水 $MgSO_4$ 干燥过程；产品的纯化除说明外均使用硅胶(200-300 mesh)的柱色谱法；所使用的硅胶，包括 200-300 目和 GF_{254} 为青岛海洋化工厂或烟台缘博硅胶公司生产。

1．化合物 **6** 的制备



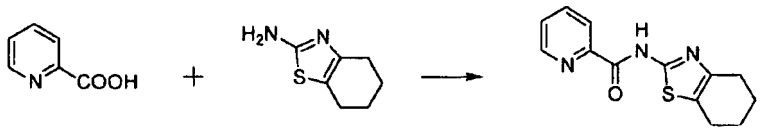
6

将邻硝基苯甲酰氯(5mmol)溶于二氯甲烷(15ml)，加入 2-氨基噻唑(500mg,5mmol)，三乙胺 (0.75ml , 5mmol)，室温下反应 8h,加入二氯甲烷稀释淬灭。将所有反应物转移至分液漏斗中，用 5%盐酸洗涤。在分液漏斗上层（水层）接近于二氯甲烷层分界处有固体漂浮。将固体用二氯甲烷洗 ，经柱色谱纯化(石油醚 ： 乙酸乙酯=3 : 1, V/V)得到白色固体产物 **6** 503mg，产率 40.4%。

^{1}H NMR (DMSO,300MHz):

δ (ppm) 8.18(d, J =8.1Hz ,1H), 7.91－7.86 (m, 1H), 7.82-7.77(m, 2H), 7.55 (d, J = 3.6Hz ,1H), 7.34 (d, J= 3.6 Hz, 1H)

2．化合物 **18** 的制法



15 **18**

将 **15**（195mg,1.26mmol）,2－羧基吡啶（156mg,1.26mmol）,DCC（270mg,1.30mmol）、DMAP（11mg,cat）的混合物中加入二氯甲烷（5ml）,氩气保护，室温下搅拌 8 小时。用乙酸乙酯稀释,过滤,滤液蒸除溶剂,残留物经柱色谱纯化(石油醚 ： 乙酸乙酯=4 : 1, V/V)得到 247mg 白色固体产物 **18**，产率 75.7%。

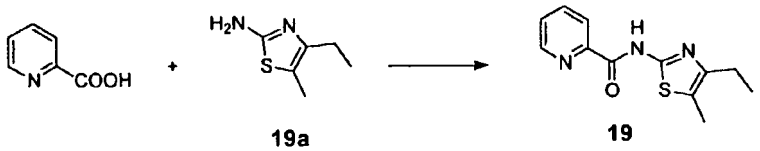
^{1}H NMR ($CDCl_3$,300MHz):

δ (ppm)11.08(s.,1H), 8.62 (d, J = 5.2Hz, 1H), 8.27 (d, J = 7.8Hz, 1H), 7.92(dt, J= 7.8,7.8,1.5 Hz, 1H), 7.51 (dd, J= 7.8,5.2 Hz, 1H), 2.72 (d, J= 13.5 Hz, 4H), 1.88(s,4H);

^{13}C NMR ($CDCl_3$, 300MHz): δ (ppm)

161.76(1C),154.61(1C),148.76(1CH),148.18(1C),145.17(1C),137.90(1CH),127.33(1CH), 123.59(1C), 122.91(1CH), 26.65(1CH2), 23.56(1CH2),23.23(2CH2);

3．化合物 **19** 的制法：



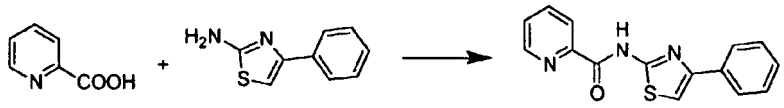
19a 19

将 **19a**（28mg,0.197mmol）,2一羧基吡啶（25mg,0.197mmol）,DCC（43mg,0.20mmol）、DMAP（cat）的混合物中加入二氯甲烷（1ml）,氩气保护，室温下搅拌 8 小时。用乙酸乙酯稀释,过滤,滤液蒸除溶剂,残留物经柱色谱纯化(石油醚 ： 乙酸乙酯=5 ： 1, V/V)，得到 14mg 白色固体产物 **19**。产率 75.7%。

^{1}H NMR ($CDCl_3$,300MHz):

δ (ppm)11.02(s.,1H), 8.61 (dd, *J* = 4.8,1.5Hz, 1H), 8.27 (d, *J* = 7.5Hz, 1H), 7.91(dt, *J*= 7.5,7.5,1.5 Hz, 1H), 7.50 (ddd, *J*= 7.5,4.8,1.5 Hz, 1H), 2.62 (q, *J*= 7.5,7.5,7.5 Hz, 2H), 2.34(s,3H), 1.22 (t, *J*= 7.5,7.5 Hz, 3H),;

4．化合物 **22** 的制备：



22a 22

将 **22a**（23mg,0.131mmol）,2－羧基吡啶（17mg,0.131mmol,1eq）,DCC（29mg,0.14mmol）、DMAP（cat）的混合物中加入二氯甲烷（2ml）,氩气保护，室温下搅拌 8 小时。用乙酸乙酯稀释,过滤,滤液蒸除溶剂,残留物经柱色谱纯化(石油醚 ： 乙酸乙酯=5: 1, V/V)得到 21mg 青色固体产物 **22**。

^{1}H NMR ($CDCl_3$,300MHz):

δ (ppm)11.25(s.,1H), 8.66 (d, *J* = 4.5Hz, 1H), 8.30 (d, *J* = 7.8Hz, 1H), 7.92(dd, *J*= 7.8,7.8 Hz, 1H), 7.89 (d, *J*= 7.8 Hz, 2H), 7.53 (dd, *J*= 7.8,4.5 Hz, 1H), 7.43 (t, *J*= 7.8,7.8 Hz, 2H), 7.33 (t, *J*= 7.8,7.8 Hz, 1H),7.22 (s,1H);

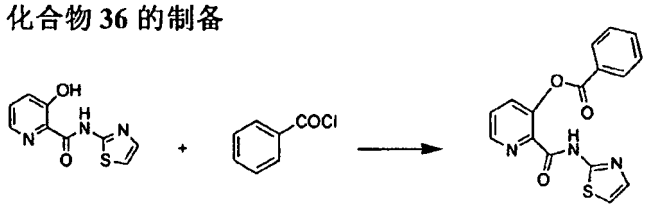
^{13}C NMR ($CDCl_3$, 300MHz): δ (ppm)

162.34(1C),157.45(1C),150.73(1C),148.86(1CH),147.98(1C),138.02(1CH),

134.63(1C),128.98(2CH),128.27(1CH),127.59(1CH),126.34(2CH),

123.08(1CH),108.21(1CH);

5．化合物 **36** 的制备



36

将 **33**(20mg,0.09mmol)溶于二氯甲烷（ 4ml ），加入三乙胺（ 0.1ml），-78℃下注入

苯甲酰氯（0.13mmol，1.5eq），保持同温下反映 3h,自然升至室温,再反应 2h,得到淡黄色溶液。抽干，溶于二氯甲烷/甲苯，经柱色谱纯化(石油醚：乙酸乙酯=3：1, V/V)得到化合物 **36**。

^{1}H NMR ($CDCl_3$,300MHz):

δ (ppm) 11.33(br.,1H), 8.60(d, *J* =4.5Hz ,1H), 8.29 (d, *J* = 7.5Hz, 2H), 7.75-7.63(m,3H), 7.56 (t, *J* = 4.5,4.5Hz ,2H), 7.50(d, *J*= 3.0Hz, 1H), 7.05 (d, *J* =3.0Hz,1H).

^{13}C NMR ($CDCl_3$, 300MHz): δ (ppm)

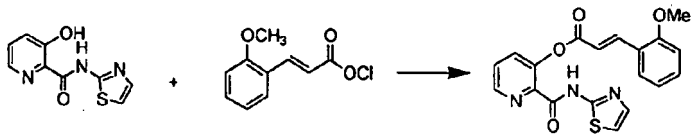
165.13(1C), 160.29(1C), 157.82(1C), 148.59(1C), 146.03(1CH), 139.88(1C),

137.87(1CH), 134.12(1CH), 133.90(1CH), 130.85(2CH), 129.13(1C), 128.88(2CH), 128.74(1CH), 113.83(1CH);

EIMS (m/z): 325(M^+, 7%) , 226(8), 197(6), 127 (16), 105(69)

97 (53) , 91 (69) , 85 (63) , 71 (100) , 69(88);

7．化合物 **44** 的合成



44

将 **33**（98mg,0.45mmol）中加入二氯甲烷（8ml),再加入三乙胺(0.1ml,68mg,0.67mmol)，然后将溶于二氯甲烷的酰氯在－78℃下加入。同温下反应 1h,然后自然升至室温，继续反应过夜。反应液淡黄色混浊。抽干，溶于二氯甲烷 / 甲苯，经柱色谱纯化(石油醚：乙酸乙酯=3：1, V/V)得到 48mg 白色固体产物，为化合物 **44**：

^{1}H NMR ($CDCl_3$,300MHz):

δ (ppm) 8.55(dd, *J* =4.2,1.5Hz ,1H), 8.25 (d, *J* = 16Hz, 1H), 7.69-7.59(m, 3H), 7.51 (d, *J* = 3.6Hz ,1H), 7.40(dt, *J*= 8.0,1.5 Hz, 1H), 7.03-6.94 (q, *J* =8.0,18Hz, 2H),6.99 (d, *J*= 3.6 Hz, 1H) , 6.89 (d, *J*= 3.6 Hz, 1H);

^{13}C NMR ($CDCl_3$, 300MHz): δ (ppm)

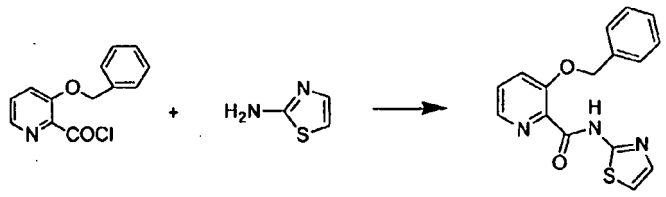
165.67(1C), 160.45(1C), 158.89(1C), 157.80(1C), 148.49(1C), 145.75(1CH),

143.47(1CH), 139.98(1C), 138.09(1CH), 133.86(1CH), 132.35(1CH), 129.76(1CH), 128.62(1CH), 123.16(1C), 120.91(1CH), 116.93(1CH), 113.75(1CH), 111.38(1CH), 55.67(1CH3);

EIMS (m/z): 381(M^+, 7%) , 353 (8), 324(16), 225 (52), 221(26)

161 (90) , 127 (28) , 123 (47) , 95 (49), 71 (60), 69 (100).

8．化合物 **47** 的制备



47

室温下在酰氯中加入二氯甲烷（2ml），再加入 2-氨基噻唑(11.4mg, 0.114mmol)，氩气保护，加入三乙胺(19ul, 0.114mmol)，反应过夜。抽干，加入二氯甲烷（10ml），用水洗（3×3ml），饱和食盐水洗(3ml). 干燥（无水 MgSO4)。过滤，滤液浓缩，溶于二氯甲烷，经柱色谱纯化(石油醚 ： 乙酸乙酯=1 ： 1, V/V)得到 6mg 红色固体产物，为化合物 47：

^{1}H NMR ($CDCl_3$,300MHz):

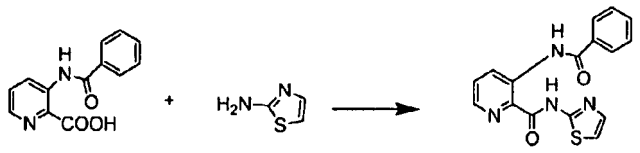
δ (ppm)8.25 (dd, J = 2.7，2.7 Hz, 1H), 7.52－7.50 (m, 3H), 7.42-7.32(m, 5H), 7.02(d, J= 3.6 Hz, 1H);

^{13}C NMR ($CDCl_3$, 300MHz): δ (ppm)

161.30(1C), 158.38(1C), 156.33(1C), 140.73(1CH), 137.80(1CH), 136.47(1C), 135.85(1C), 129.06(2CH), 128.54(1CH), 128.42(1CH), 127.12(1CH), 127.06(1CH), 123.50(1CH), 113.64(1CH), 71.14(^{1}CH$_2$);

EIMS (m/z): 311(M^+, 6%) , 220 (7), 197(8), 189 (43), 123 (19) , 111 (29) , 97 (44) , 91(100). 85 (55), 71(83), 69 (78).

9．化合物 51 的制备



51a

51

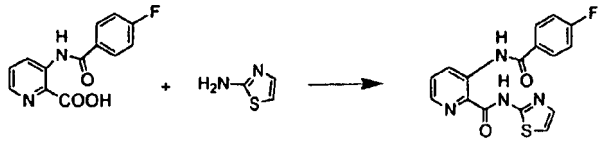
将 51a （ 40mg, 0.165mmol ） 中 加 入 DCC （ 41mg, 0.198mmol ）、 DMAP（10mg, 0.083mmol, 0.5eq)、活化的 4A 分子筛(100mg)，氩气保护下，注入重蒸甲苯(1ml)，室温搅拌 20min, 加入 2 氨基噻唑（17mg, 0.165mmol)，于 70℃下搅拌 1.5 小时。过滤，滤液直接上柱，经柱色谱纯化(石油醚 ： 乙酸乙酯=3 : 1, V/V)得到白色固体产物 4mg。

^{1}H NMR ($CDCl_3$, 300MHz):

δ (ppm) 9.40 (dd, J = 8.4,1.5Hz,1H), 8.36(dd, J = 4.5 Hz, 1H), 8.12-8.09 (m, 2H), 7.62-7.55 (m, 5H), 7.09 (d, J= 3.6 Hz, 1H);

EIMS (m/z): 324 (M^+, 7), 239(6), 225 (19), 197 (11), 125 (25), 121 (11) 111(39), 97 (60), 85 (65), 71 (100), 69 (96).

10. 化合物 53 的制备



53a 53

将 53a（75mg,0.288mmol）中加入 DCC（77mg,0.375mmol,1.3eq）、DMAP（18mg,0.144mmol,0.5eq）、活化的 4A 分子筛（100mg），氩气保护下，注入重蒸甲苯（1ml），室温搅拌 20min,加入 2-氨基噻唑（29mg,0.288mmol），70℃下搅拌 3 小时。过滤，滤液直接上柱，经柱色谱纯化(石油醚 ：乙酸乙酯=1：1, V/V)得到 13mg 白色固体产物,化合物 53。

^{1}H NMR ($CDCl_3$,300MHz):

δ (ppm)12.49 (s, 1H), 11.38(br.,1H), 9.36 (dd, *J* = 8.4,0.9Hz, 1H), 8.36(dd, *J*= 4.5,0.9 Hz, 1H), 8.14-8.10 (m, 2H), 7.61-7.57 (m, 2H), 7.28-7.22 (m, 2H),7.10 (d, *J*= 3.6 Hz, 1H);

^{1}H, ^{1}H –COSY NMR(CDCl3,300MHz):

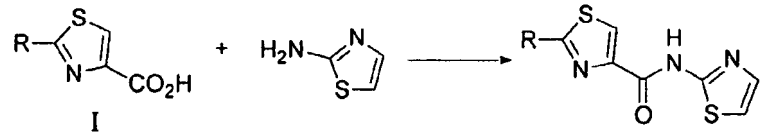
相关峰：CH-CH-CH , CH(2H)-CH(2H) , CH-CH;

^{13}C NMR ($CDCl_3$, 300MHz): δ (ppm)

167.27(1C), 165.53(1C), 165.12(1C), 163.91(1C), 157.06(1C), 142.79(1CH), 139.47(1C), 138.62(1CH), 131.52(1C), 130.33(1CH), 130.21(1CH),129.17(1CH), 129.04(1CH), 116.45(1CH), 116.16(1CH),114.46(1CH);

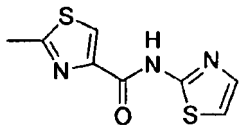
11、化合物 55-59 的制备

化合物 55-59 的制备按下列通式进行：



I

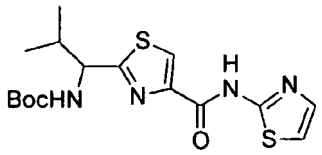
将 I（1mmol），2-氨基噻唑（120mg, 1.2mmol），HOBT（162mg, 1.2mmol），EDC(230mg, 1.2mmol), 少量 4 Å 分子筛的混合物中注入 10mL 重蒸 DMF，rt 搅拌反应过夜。用乙酸乙酯稀释,过滤,水洗三次除去 DMF，饱和食盐水洗，有机相无水 $MgSO_4$ 干燥。旋转蒸除溶剂，残留物经柱色谱纯化。



55

化合物 55： ^{1}H NMR (CDCl3,300MHz): δ (ppm): 11.08(br, 1H), 8.12(s, 1H), 7.53(d, J=3.6Hz, 1H), 7.00(d, J=3.6Hz, 1H), 2.70(s, 3H);13C NMR (CDCl3, 300MHz): δ (ppm) 166.87(1C), 158.54(1C), 158.04(1C), 147.70(1C), 138.30(1CH), 125.59(1CH), 113.79 (1CH),

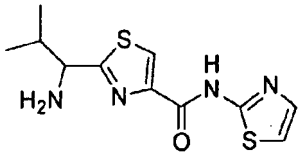
19.25(1CH3);



56

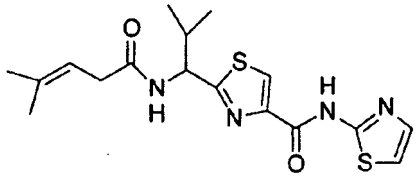
化合物 56：^{1}H NMR (CDCl3,300MHz): δ (ppm)：10.64(br, 1H), 8.20(s, 1H), 7.49(d, J=3.6Hz, 1H), 7.02(d, J=3.6Hz, 1H), 5.30(d, J=8.7Hz, 1H), 4.89(m, 1H), 2.32(m, 1H), 0.97(d, J=6.9Hz, 1H), 0.91(d, J=6.9Hz, 1H);

^{13}C NMR (CDCl3, 300MHz): δ (ppm) 173.84(1C), 158.31(1C), 157.78(1C), 155.55(1C), 147.97(1C), 138.10(1CH), 125.36(1CH), 114.14(1CH), 80.52(1C), 58.10(1CH), 33.39(1CH), 28.44(3CH3), 19.41(1CH3), 17.59(1CH3);



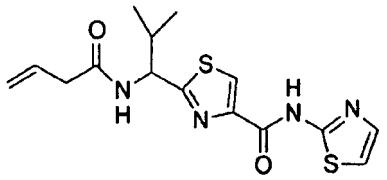
57

化合物 57：^{1}H NMR (CDCl3,300MHz) δ (ppm)：8.66(br, 2H), 7.63(d, J=3.9Hz, 1H), 7.21(d, J=3.9Hz, 1H), 4.86(m, 1H), 2.50(m, 1H), 1.11(d, J=6.9Hz, 1H), 0.98(d, J=6.9Hz, 1H);



58

化合物 58：^{1}H NMR (CDCl3,300MHz) δ (ppm)：8.22(s, 1H), 7.51(d, J=3.6Hz, 1H), 7.05(d, J=3.6Hz, 1H), 6.60(d, J=8.7Hz, 1H), 5.38(m, 1H), 5.24(dd, J=8.7,5.7Hz, 1H), 3.06(d, J=7.8Hz, 2H), 2.38(m, 1H), 1.82(s, 3H), 1.69(s, 3H), 0.88(d, J=6.9Hz, 3H), 0.86(d, J=6.9Hz, 3H);

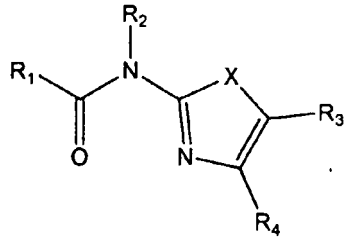


59

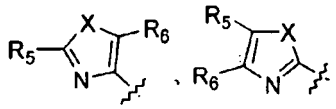
化合物 59：^{1}H NMR (CDCl3,300MHz) δ (ppm)：8.22(s, 1H), 7.50(d, J=3.6Hz, 1H), 7.05(d, J=3.6Hz, 1H), 6.77(d, J=8.7Hz, 1H), 6.00(m, 1H), 5.26(m, 3H), 3.12(d, J=6.9Hz, 2H), 2.33(m, 1H), 0.97(d, J=6.6Hz, 3H), 0.96(d, J=6.6Hz, 3H)

权 利 要 求 书

1．一类结构式如下的甲硫氨酰氨肽酶抑制剂



其中 R_1 为 C_1-C_4 的烷基、取代烷基、C_3-C_6 的环烷基、取代环烷基、芳基、吡啶基；由 C_1-C_4 的烷基、硝基、羧基、醛基、烷氧基、胺基、酰 氨基、巯基的取代芳基、取代吡啶基和具有如下结构的杂环或取代杂环



R_5、R_6 为 H、C_1-C_4 的烷基、取代烷基、C_3-C_6 的环烷基、取代环烷基、芳基、吡啶基、硝基、羧基、醛基、烷氧基、胺基、酰氨基、巯基的取代芳基、取代吡啶基；

R_2 为 H、C_1-C_4 烷基、取代烷基、芳基、由 C_1-C_4 的烷基硝基、羧基、醛基、烷氧基、胺基、酰 氨基、巯基的取代芳基；

R_3 为 H、C_1-C_4 烷基、取代 C_1-C_4 烷基、卤素；芳基、取代芳基；

R_4 为 H、C_1-C_4 烷基、取代烷基、取代芳基；

X 为 O、S、N、杂原子。

2．根据权利要求 1 所述的甲硫氨酰氨肽酶抑制剂，其特征在于：

当 R_1 为吡啶、取代吡啶包括卤素、酰胺、烷氧基、羟基、羧基、酯基、醚时，

R_2 为 H；

R_3 为 H、Br 、烷基；

R_4 为 H、烷基、取代芳基。

3．根据权利要求 1 所述的甲硫氨酰氨肽酶抑制剂，其特征在于：

当 R_1 为芳基、取代芳基包括硝基、胺基、C_1-C_4 的烷氧基、羟基、羧基、苄基时

R_2 为 H；

R_3 为 H、卤素、C_1-C_4 烷基；

R_4 为 H、C_1-C_4 烷基、取代芳基。

4．根据权利要求 1 所述的甲硫氨酰氨肽酶抑制剂，其特征在于：

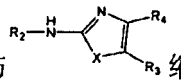
当 R_1 为杂环或取代杂环时

R_2 为 H

R_3 为 H

R_4 为 H

R_5、R_6 为 H、C_1-C_4 的烷基、取代烷基、C_3-C_6 的环烷基、取代环烷基、芳基、吡啶基、硝基、羧基、醛基、烷氧基、胺基、酰 氨基、巯基的取代芳基、取代吡啶基；

5. 如权利要求 1 所述的甲硫氨酰氨肽酶抑制剂的制备方法，其特征在于由 Y 为羟基、卤素或其他活性基团的 R1COY 与  缩合。

6. 根据权利要求 4 所述的甲硫氨酰氨肽酶抑制剂的制备方法其特征在于缩合剂为 DCC、EDC、DIC、HBTU。

7. 根据权利要求 4 所述的甲硫氨酰氨肽酶抑制剂的制备方法其特征在于缩合反应溶剂为二氯甲烷、二甲基呋喃、二氯乙烷、甲苯、苯、水、二氧六环或上述溶剂的混合溶剂。

8. 根据权利要求 4 所述的甲硫氨酰氨肽酶抑制剂的制备方法其特征在于反应温度为 -20°C 至室温或加热温度从 50°C 至 130°C。

9. 根据权利要求 4 所述的甲硫氨酰氨肽酶抑制剂的制备方法其特征在于缩合反应时加入活化剂 HOBT、五氟苯酚或分子筛。

10. 根据权利要求 4 所述的甲硫氨酰氨肽酶抑制剂的制备方法其特征在于缩合反应时用三乙胺、二乙丙基乙基胺、吡啶、DMAP 碱作催化剂。

11. 如权利要求 1 所述甲硫氨酰氨肽酶抑制剂作为抗肿瘤或抗感染药物先导化合物。

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN03/00213

A. CLASSIFICATION OF SUBJECT MATTER

C07D277/22 277/38 263/30 263/48 207/30 207/34 401/12 403/12 405/12 407/12 417/12 A61K31/40 31/41

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99/57098 A (11 Nov.1999, the whole doc.)	1-11
X	CN 1033626 A(5 July 1999, the whole doc.)	1-11
X	CN 1035826 A (27 Sep. 1989, the whole doc.)	1-11

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim (S) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
June 16, 2003	24 JUL 2003 (24.07.03)
Name and mailing address of the ISA/CN 6 Xitucheng Rd., Jimen Bridge, Haidian District, 100088 Beijing, China Facsimile No. 86-10-62019451	Authorized officer Zhou Hubo Telephone No. 86-10-62093075

Form PCT/ISA /210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN03/00213

Box I Observations where certain claims were found unsearch able (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos: 1(part)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

In claim 1 the terms such as "substituted aryl" define too broad a scope that it is impossible for the examiners to do a completed search, so the search is mainly based on the related embodiment of description (in which X=S).

3. ☐ Claims Nos:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant,this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant.Consequently ,this international search report is restricted to the invention first mentioned in the claims;it is covered by claims Nos.:

Remark on protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN03/00213

WO 99 57098 A	19991111		
		AU3877899 A	19991123
		NO200005506 A	20001229
		EP1073633 A2	20010207
		CZ200003967 A3	20010314
		SK200001628 A3	20010510
		KR2001043198 A	20010525
		CN1308608 A	20010815
CN 1033626 A	19890705	EP0296673 A	19881228
		AU1832988 A	19890105
		JP1026572 A	19890127
		DK8803467 A	19881226
		US4877802 A	19891031
		SU1579458 A	19900715
		EP0296673 B1	19940309
		DE3888245G G	19940414
		CA1328867 C	19940426
		ES2061628T T3	19941216
		DK169743B B	19950213
CN 1035826 A	19890927	EP0313091 A	19890426
		AU2414788 A	19890427
		BR8805475 A	19890704
		JP1230563 A	19890914
		HU49595 A	19891030
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		JP2067207 A	19900307
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		RO107654 B1	19931230
		RO107655 B1	19931230
		JP2648621B2 B2	19970903
		JP2723155B2 B2	19980309
		JP2788458B2 B2	19980820

国际检索报告

国际申请号
PCT/CN03/00213

A. 主题的分类
C07D277/22 277/38 263/30 263/48 207/30 207/34 401/12 403/12 405/12 407/12 417/12 A61K31/40 31/41
按照国际专利分类表(IPC)或者同时按照国家分类和IPC两种分类

B. 检索领域

检索的最低限度文献(标明分类体系和分类号)
IPC 7 C07D A61K

包含在检索领域中的除最低限度文献以外的检索文献

在国际检索时查阅的电子数据库(数据库的名称和，如果实际可行的，使用的检索词)

C. 相关文件

类 型*	引用文件，必要时，指明相关段落	相关的权利要求编号
X	WO 99/57098 A (1999年11月11日，见全文)	1－11
X	CN 1033626 A(1989年7月5日，见全文)	1－11
X	CN 1035826 A(1989年9月27日，见全文)	1－11

☐ 其余文件在C栏的续页中列出。 ☒ 见同族专利附件。

* 引用文件的专用类型：
"A" 明确叙述了被认为不是特别相关的一般现有技术的文件
"E" 在国际申请日的当天或之后公布的在先的申请或专利
"L" 可能引起对优先权要求的怀疑的文件，为确定另一篇引用文件的公布日而引用的或者因其他特殊理由而引用的文件
"O" 涉及口头公开、使用、展览或其他方式公开的文件
"P" 公布日先于国际申请日但迟于所要求的优先权日的文件
"T" 在申请日或优先权日之后公布的在后文件，它与申请不相抵触，但是引用它是为了理解构成发明基础的理论或原理
"X" 特别相关的文件，仅仅考虑该文件，权利要求所记载的发明就不能认为是新颖的或不能认为是有创造性
"Y" 特别相关的文件，当该文件与另一篇或者多篇该类文件结合并且这种结合对于本领域技术人员为显而易见时，权利要求记载的发明不具有创造性
"&" 同族专利成员的文件

国际检索实际完成的日期	国际检索报告邮寄日期
2003年6月26日	24.7月 2003 (24.07.03)
国际检索单位名称和邮寄地址 ISA/CN 中国北京市海淀区西土城路6号(100088) 传真号： 86-10-62019451	受权官员 周胡斌 电话号码： 86-10-6209[illegible]

国际检索报告

国际申请号
PCT/CN/03/00213

第I栏　关于某些权利要求不能作为检索主题的意见(接第 1 页第 1 项)

按条约 17(2)(a)对某些权利要求未作国际检索报告的理由如下：

1. ☐ 权利要求（编号）：
因为它们涉及到不要求本国际检索单位检索的主题，即：

2. ☒ 权利要求（编号）：1（部分）
因为它们涉及到国际申请中不符合规定的要求的部分，以至于不能进行任何有意义的国际检索，
具体地说：
由于权利要求 1 中诸如“取代的”之类的术语限定了一个过宽的保护范围，使得审查员不可能进行完全的检索，所以本检索主要是针对权利要求 1 中说明书实施例主要涉及的部分即 X＝S 时的情况进行检索的。

3. ☐ 权利要求（编号）：
因为它们是从属权利要求，并且没有按照细则 6.4(a)第 2 句和第 3 句的要求撰写。

第II栏　关于缺乏发明单一性时的意见(接第 1 页第 2 项)

本国际检索单位在该国际申请中发现多项发明，即：

1. ☐ 由于申请人按时缴纳了所要求缴纳的全部附加检索费，本国际检索报告针对全部可作检索的权利要求

2. ☐ 由于无需付出有理由要求附加费的劳动即能对全部可检索的权利要求都进行检索，本国际检索单位未通知缴纳任何附加费。

3. ☐ 由于申请人仅按时缴纳了部分所要求缴纳的附加检索费，本国际检索报告仅涉及已缴费的那些权利要求，具体地说，是权利要求（编号）：

4. ☐ 申请人未按时缴纳所要求的附加检索费。因此，本国际检索报告仅涉及权利要求中首先提到的发明：
包含该发明的权利要求是（编号）：

关于异议的说明：☐ 申请人的异议书随附加检索费同时提交。
☐ 支付附加检索费时未提交异议书。

国际检索报告

关于同族专利成员的情报

国际申请号 PCT/CN03/00213

检索报告中引用的专利文件	公布日期	同族专利成员	公布日期
WO 99 57098 A	19991111		
		AU3877899 A	19991123
		NO200005506 A	20001229
		EP1073633 A2	20010207
		CZ200003967 A3	20010314
		SK200001628 A3	20010510
		KR2001043198 A	20010525
		CN1308608 A	20010815
CN 1033626 A	19890705	EP0296673 A	19881228
		AU1832988 A	19890105
		JP1026572 A	19890127
		DK8803467 A	19881226
		US4877802 A	19891031
		SU1579458 A	19900715
		EP0296673 B1	19940309
		DE3888245G G	19940414
		CA1328867 C	19940426
		ES2061628T T3	19941216
		DK169743B B	19950213
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		RO107654 B1	19931230
		RO107655 B1	19931230
		JP2648621B2 B2	19970903
		JP2723155B2 B2	19980309
		JP2788458B2 B2	19980820

Translation from Chinese

(Page 1)

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization

International Bureau

(43) International Publication Date

09 October, 2003 (09.10.2003)

(10) International Publication Number

WO 03/082838 A1

(51) International Patent Classification⁷: C07D 277/22, 277/38, 263/30, 263/48, 207/30, 207/34, 401/12, 403/12, 405/12, 407/12, 417/12, A61K 31/40, 31/41

(21) International Application Number: PCT/CN03/00213

(22) International Filing Date: 25, March, 2003 (25.03.2003)

(25) Application Language: Chinese

(26) Publication Language: Chinese

(30) Privileges:

02111230.4 April 02, 2002 (02.04.2002) CN

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(81) Designated States (Country): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW

(84) Desig. Counts. (Regs): ARIPO Patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian Patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European Patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI Patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

Declaration according to Rule 4.17:

— As to the applicant's entitlement, as of the international filing date, to apply for and be granted a patent (Rule 4.17(ii)) to all designated states except the USA

— As to the applicant's entitlement, as of the international filing date, to apply for and be granted a patent (Rule 4.17 (ii)) to the following state: the USA

— As to the applicant's entitlement, as of the international filing date, to claim priority for early application (Rule 4.17 (iii)) to all designated states except the USA

— As to the applicant's entitlement, as of the international filing date, to claim priority for early application (Rule 4.17 (iii)) to the following state: the USA

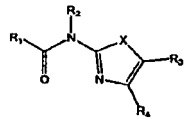
— Inventorship (Rule 4.17 (iv)) USA only

This International Publication:

— with international search report.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" which appears at the beginning of each regular issue of the PCT Gazette".

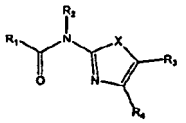
(54) Title: NEW METHIONINE AMINOPEPTIDASE INHIBITOR



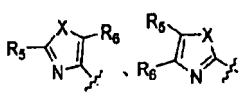
(I)

(57) Abstract: The invention provides a new methionine aminopeptidase inhibitor with the following formula (I): wherein R_1, R_2, R_3, R_4, R_5, R_6, and X have the meanings given in the description. Pharmacological tests show that these display good inhibition activity for methionine aminopeptidase. [See the continuous page]

(57) **Abstract:** The invention provides a new type of methionine aminopeptidase inhibitor with the following formula:



Wherein R_1 represents alkyl at C_1-C_4, alkyl substituent, cycloalkyl at C_3 to C_6, cycloalkyl substituent, aryl, pyridyl; aryl substituted with alkyl at C1-C4, nitryl, carboxyl, aldehyde group, alkoxyl, amidocyanogen, amide group, hydrosulfide group, pyridyl substituent, and heterocycle or substituent heterocycle with the following structure[1]:



R_5, R_6 represent H, alkyl at C_1-C_4, alkyl substituent, cycloalkyl at C_3-C_6, cycloalkyl substituent, aryl, pyridyl, nitryl, carboxyl, aldehyde group, alkoxyl, amidocyanogen, amide group, aryl substituted by mercapto, pyridyl substituent;

R_2 represents H, alkyl at C1-C4, alkyl substituent, aryl, aryl substituted by alkyl at C1-C4, nitryl, carboxyl, aldehyde group, alkoxyl, amidocyanogen, amide group and hydrosulfide group;

R_3 represents H, alkyl at C1-C4, alkyl substituent at C1-C4, halogen; aryl, aryl substituent;

R_4 represents H, alkyl at C1-C4, alkyl substituent, aryl substituent;

X represents O, S, N, hetero atoms.

Pharmacological tests show that they display good inhibition activity for methionine aminopeptidase.

[1] Translator's note: the original source is grammatically odd here

Translation from Chinese

(Page 3)

A type of methionine aminopeptidase inhibitor

Technical Scope

This invention involves a type of small molecule organic compound that manifests high inhibition activity for methionine aminopeptidase (MetAPs), and certain selectivity for different subtypes of MetAPs. This type of compound may therefore be employed as a new leading compound for research into anti-cancer and antibiotic drugs.

Technical Background

In the cytoplasm of all prokaryotic cells, all protein translation starts from the methionine (Met) at the N terminal, however, in the eukaryotic cells and in chondriosome and chlorophyl bodies, the protein translation starts from N-formylmethonine (fMet), and the formyl group is generally removed in the translation process by the enzyme peptide deformylase (Pdf). In both cases, MetAPs selectively remove the new produced protein or the Met at the N terminal of polypeptide chain. Such removals are crucial to the proteins' post-translational modification, precise positioning and functioning in cells.

The first MetAPs discovered was Escherichia coli MetAP (EcMetAP); enzymes with similar structure and functions were discovered in saccharomyces and mammals. Based on the sequence comparison, the C terminals of these MetAPs are highly homologous, but there are prolongations at the N terminal with structures similar to the zinc-finger structure. These sequences were later experimentally proved to be sequences connected to ribosome, which do not affect the proteolytic enzyme activity (J. Biol. Chem. 1990; 265: 19892-19897). This type of enzymes and EcMetAP were classified as MetAP I; later, another MetAP was isolated & purified and cloned from pig liver (J. Biol. Chem. 1992; 267: 20667-20673). Sequence comparison indicate that the C terminal of this new MetAP is less homologous to EcMetAP than that of the MetAP I (as it has a sequence consisting of approximately 60 aminophenol inserted at the C terminal), and this new MetAP's N terminal is a sequence in the structure of zinc-finger substituted by acid or neutral poly aminophenol residues (this structure has been proved to be an inhibitor for phosphorylation of the protein translation initiator in the eukaryotic cells, which does not affect the proteolytic enzyme activity). In order to denote its difference from MetAP I, this type of enzyme was classified as MetAP II, such as the MetAPs II successively found in saccharomyces, drosophilae, mice and human beings.

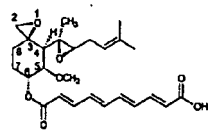
MetAPs widely exist in the cells of various forms of life in nature, and has a certain selectivity to the substrate. Experiments show that regardless of whether the substrate is intrinsic protein, extrinsic recombined and expressed protein, or polypeptide (Biochemistry, 1987; 26: 8242-8246), the substrate can be enzymatic hydrolysed by MetAPs only when the second aminophenol (P'_1) after the N terminal Met of the protein or polypeptide substrate is a non-polarized chain under 3.68 Å. It is currently believed that the MetAPs can function as long as the aminophenol at P'_1 position is one of the following 7 aminophenols: Gly, Ala, Ser, Thr, Pro, Val and Cys (J. Bacteriol. 1987; 169:751-757; Biochemistry. 1999; 38: 14810-14819).

The basic function of MetAPs in cells is to remove the N terminal Met that newly combines protein, thus laying a foundation for the following functions of the protein. The removal of new protein or the N terminal Met of the polypeptide chain is crucial to the post-translational modification, precise position in cell, direct degradation (J. Biol. Chem. 1982; 257: 3532-3538) and appropriate functioning of the protein.

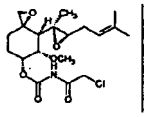
Currently, the MetAP inhibitors can be classified as covalent combination inhibitors and non-covalent combination inhibitors according to their function patterns. Besides, there is another type of inhibitors which are transition-state analog and reaction products, which have similar inhibition mechanisms to the Bestatin analog (Biochemistry. 1999; 38: 14810-14819).

Fumagillin and its derivative TNP-470 (IC_{50} at nanomolar) are a type of drug that can specifically inhibit endothelial cells growth effectively to inhibit the chemical compound of MetAPs by covalent combination. Research shows that human MetAP II (hMetAP II) is the target for intracellular fumagillin and TNP-470, such combinations inhibit the activity of this protein enzyme by covalent modification of the conservative aminophenol residue His at the catalysis site (Proc. Natl. Acad. Sci. USA. 1998; 95: 15183-15188).

Non-covalent inhibitor is a substrate analog inhibitor modified using a Bestatin template, an effective inhibitor of leucine aminopeptidase (LAP) enzyme, targeting the substrate properties of MetAPs. The result of replacement at P_1 and P_1' positions of Bestatin with isoleucine and Alanine respectively is an unhydrolyzable substrate analog with its inhibition activity IC_{50} at 5μM, the best eMetAP inhibitor ever reported.

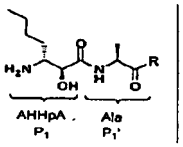


Fumagillin



TNP-470

Covalent combination inhibitors of MetAPs



R = Leu–Val–Phe-Ome

Non-covalent inhibitors of eMetAPs

In recent years, considerable progress has been achieved in the chemical treatment of cancer, and the survival time of the patients has been obviously prolonged. In particular, breakthroughs have been made in the treatment of Leukemia and Lymphoma, etc. However, satisfactory results have not been achieved in the treatment of solid tumours, the

most severe type of tumour that endangers the health of human beings, which includes 90% of all malignant tumours. Pharmacologists and tumour specialists have gradually come to the deep understanding that, for the improvement of tumour treatment, a breakthrough can only be achieved through research focusing on the mechanism of the formation and development of tumours. The anti-cancer drugs under development range from traditional cytotoxic drugs to mechanism-oriented new drugs that function with multiple links. The tumour vascular system is a new and hopeful target for anti-cancer treatment, as the growth and metastasis both rely on the formation of new vessels (angiogenesis). Through the tumour vessels, the tumour can not only obtain nutrition and oxygen from its host, but also transport cells to its host and maintain its growth and induce angiogenesis in other places of the body, eventually realizing tumour metastasis. Nowadays, one of the most active anti-cancer pharmaceutical research fields is to control tumour formation through angiogenesis inhibition[2]. The anti-tumour capabilities of fumagillin and its analogs operate through their effect on the vascular system of the tumour, in which tumour growth is controlled through specific inhibition of vascular endothelial cell growth. This indicates that MetAPs, especially hMetAP II, can be employed as a function target for a new type of anti-angiogenesis drugs, on which the effective and specific inhibitors can be employed as new anti-cancer drug.

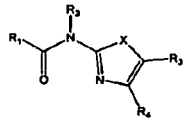
The knock-out experiment using $MetAP_1$ and MetAPs gene only in prokaryotic life forms shows that all the coliform, typhoid salmonella and saccharomyces are unable to continue growth, which indicates that $MetAP_1$ is crucial to the growth process of prokaryotic life forms. Therefore, chemical compounds that can selectively inhibit $MetAP_1$ may be employed as new anti-bacterial infection drugs.

Content of Invention

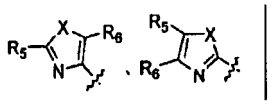
Invention Objective: This invention designs and synthesizes new small molecular organic compounds as inhibitors for MetAPs, and carries out in-depth research into the relationship between their structures and activities, and discovers the leading compounds for anti-cancer and anti-infection drugs at the same time as it explains the functional mechanisms of MetAPs in pathological terms.

The other objective of this invention is to provide the synthesis method for this type of compounds.

The MetAPs inhibitors described in this invention have the following formula:



wherein R_1 represents alkyl at C1-C4, alkyl substituent, cycloalkyl at C_3-C_6, cycloalkyl substituent, aryl, pyridyl; aryl substituted by alkyl at C1-C4, nitryl, carboxyl, aldehyde group, alkoxyl, amidocyanogen, amide group, sulfhydryl group, pyridyl substituent, heterocycle or substituent heterocycle with the following structure: aryl, pyridyl



[2] Translator's note: The original source has a very obvious misprint here, which should read "通过抑制肿瘤血管生成来抑制肿瘤生成..."

R_5, R_6 represent H, alkyl at C1-C4, alkyl substituent, cycloalkyl at C_3-C_8, cycloalkyl substituent, aryl, pyridyl, nitryl, carboxyl, aldehyde group, alkoxyl, amidocyanogen, amide group, aryl substituted of mercapto, pyridyl substituent;

R_2 represents H, alkyl at C1-C4, alkyl substituent, aryl, aryl substituted by alkyl at C1-C4, nitryl, carboxyl, aldehyde group, alkoxyl, amidocyanogen, amide group or mercapto;

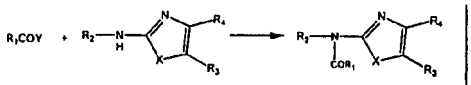
R_3 represents H, alkyl at C1-C4, alkyl substituent at C1-C4, halogen; aryl, aryl substituent;

R_4 represents H, alkyl at C1-C4, alkyl substituent, aryl substituent;

X represents O, S, N, hetero atoms.

This invention is implemented through the following process:

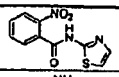
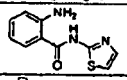
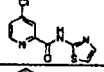
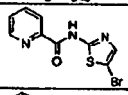
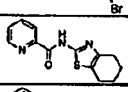
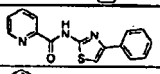
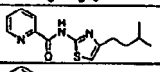
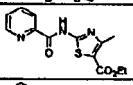
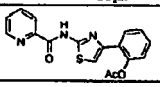
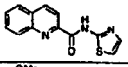
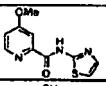
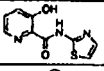
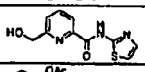
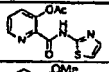
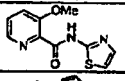
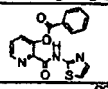
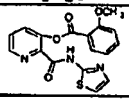
Using the chemical reaction formula:

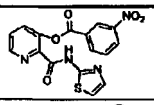
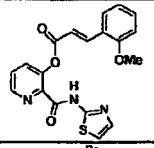
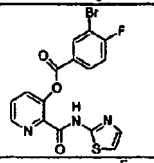
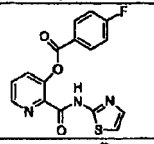
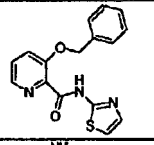
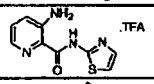
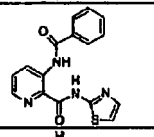
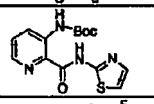
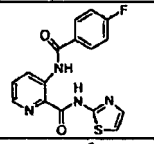
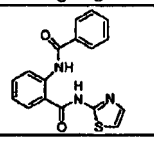
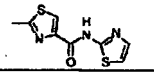


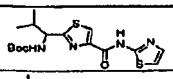
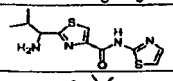
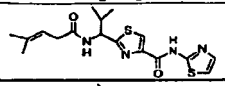
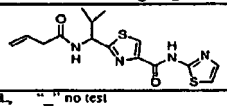
I II III

Compounds I and II are condensation polymerised into compound III, in which Y represents OH, Cl or another active group, the condensation polymerisation reaction between compounds I and II is carried out in the following solvent: CH_2Cl_2, DMF, CH_2ClCH_2Cl, toluene, benzene, water, dioxane or mixture solvent when necessary, such as CH_2Cl_2/ DMF(1:1 V/V). According to the properties of compounds, the condensation polymerisation reagents may be chosen from DCC, ECD, DIC and HBTU, etc. If necessary, a small volume of activator such as HOBT, pentafluoro phenol or a molecular sieve, etc. may be used in the reaction. Sometimes the reaction requires an alkaline catalyst such as triethylamine, diethyl propyl ethylamine, pyridine, DMAP. The reaction temperature ranges from –20°C to room temperature, but heating (generally between 50°C and 130°C) is also needed in some situations. The reaction duration also varies depending on the active groups of the reaction compounds, for instance, the reaction may be completed within a few minutes when Y is Cl, while other reactions may take longer. TLC is usually employed to determine the completion degree of the reaction. Upon completion of the reaction, extraction is generally made with solvents such as ethyl acetate, dichloromethane, chloroform, etc., which are washed with 5% HCl, water and saturated salt water solution successively. After desiccation, the solvent is removed under low temperature and pressure and the end product III is acquired through column chromatography of the concentrated solution. The production ratio ranges from 20% to 95% depending on the properties of compounds I and II. The end product is then proved using methods such as NMR.

Compound II can be synthesized according to the methods introduced in J. Org. Chem. 63, 196-200 (1998).

	Chemical Formula	$IC_{50}(\mu M)$	
		EMetAP	hMetAP1
6		NA	–
7		30% inhibition at 20μg/ml	–
16		4.84	–
17		6.0	–
18		NA	–
22		NA	–
23		NA	–
24		NA	–
25		NA	–
27		51.0	–
30		9.22	–
31		2.33	–
32		5.82	–
34		8.20	29.0
35		4.49	20.9
36		1.10	28.7
37		1.90	15.4

41		1.91	7.3
44		1.80	–
45		1.35	17.4
46		1.31	7.0
47		5.31	7.8
50		5.56	11.0
51		0.14	–
52		1.25	–
53		0.05	–
54		NA	–
55		0.4	

56		NA	–
57		NA	–
58		NA	–
59		NA	–

NA: no inhibition at 20 μg/mL, "–" no test

Bioactivity Test

A large volume of eMetAP protein is cloned and expressed using a coliform system, followed by deposition with saturated $(NH_4)_2SO_4$ solution and Q-Sepharose column chromatography and purification, after which apo-eMetAP is acquired. After incubation with Co^{2+} at appropriate concentration, a highly active enzyme may be acquired for the selection of this enzyme inhibitor.

Test Mechanism of Drug Selection Mode

The eMetAP may hydrolyse and compound the thioester bond of substrate Met-S-C-Phe. The product Met-SH then reacts with excessive DTNB, and produces 3-carboxyl-4-nitryl thiophnol salt, which demonstrates spectra absorption at 412 nm (ε 412= 13600 M-1. cm-1). The enzyme activity can be determined by its spectra absorption at 412 nm in the Spectra MAX 340 test.

Operation Process:

Regular Selection Method (Anal Biochem. 2000, 280: 159-65)

Preliminary selection is conducted among the compound solutions at three different concentrations, namely; 2 μ g/mL, 20 μ g/mL and 100 μ g/mL, when the inhibition activity is higher than 50%, 8 solutions of different concentrations are selected, and this active compound IC_{50} is tested.

Advantages of the Invention:

This invention indicates that these combined chemical compounds are a type of completely newly structured MetAP inhibitor. Compared with the existing inhibitors of this type of enzyme, these compounds feature relatively simpler structures and can be easily produced. In addition, some of these compounds demonstrate the best inhibition activity for eMetAP to date.

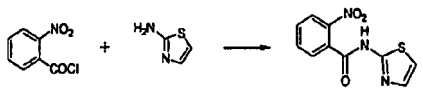
Implementation Method Specification

This invention is further explained with specific implementation cases as follows, but these explanations do not represent the entire scope of this invention.

^{1}H-NMR determined with a Varian Mercury AMX 300 instrument; MS determined with a VG ZAB-HS or VG-7070 instrument, both from EI source (70 ev) unless specifically noted; all solvents have been redistilled before use, all employed anhydrous solvents are acquired through standard desiccation methods; unless

specifically noted, all reactions are protected with Ar gas and TLC traced, all post-treatments are conducted after washing with saturated salt water solution and desiccation in anhydrous MgSO; all purifications of the product use the silicon gel (200- 300 mesh) column spectra method; the silicon gel employed, including that of 200-300 mesh and GF_{254}, is produced by Qingdao Haiyang Chemical Co., Ltd. or Yantai Yuanbo Silicon Gel Co., Ltd.

1. Production of Compound 6



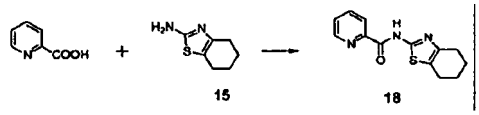
6

Dissolve 5mmol o-nitrobenzoyl chloride into 15 ml dichloromethane, add 2-aminothiazole (500 mg, 5mmol), Triethylamine (0.75 ml, 5mmol), react for 8 hours at room temperature, dilute and quench with dichloromethane. Transfer all reaction products into a separating funnel and wash with 5% HCl. Solid matter can be found floating in the upper layer (water layer) near to its separation border to the dichloromethane layer. After washing with dichloromethane and purifying with column spectra (petroleum ether: ethyl acetate= 3:1, V/V), 503 mg of product 6, the white solid matter, is obtained. The production ratio is 40.4%.

^{1}H-NMR (DMSO, 300MHz):

δ(ppm) 8.18 (d, J = 8.1 Hz, 1H), 7.91- 7.86 (m, 1H), 7.82- 7.77 (m, 2H), 7.55 (d, J = 3.6Hz, 1H), 7.34 (d, J = 3.6 Hz, 1H)

2. Production of Compound 18



15 18

Add dichloromethane (5 ml) to a mixture of compound 15 (195 mg, 1.26 mmol), 2- carboxyl pyridine (156 mg, 1.26 mmol) and DCC (270 mg, 1.30 mmol), DMAP (11 mg, cat), and stir for 8 hours at room temperature under Ar gas protection. Dilute with ethyl acetate, filtrate, and remove the solvent by distillation, purify the residue with column spectra (petroleum ether: ethyl acetate= 4:1, V/V), and 247 mg white solid product 18 is obtained. The production ratio is 75.7%.

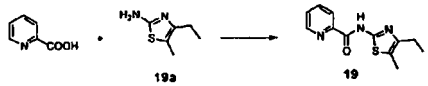
^{1}H-NMR ($CDCl_3$, 300MHz):

δ (ppm) 11.08 (s., 1H), 8.62 (d, J = 5.2 Hz, 1H), 8.27 (d, J = 7.8 Hz, 1H), 7.92 (dt, J = 7.8, 7.8, 1.5 Hz, 1H), 7.51 (dd, J = 7.8, 5.2 Hz, 1H), 2.72 (d, J = 13.5 Hz, 4H), 1.88 (s, 4H);

^{13}C NMR ($CDCl_3$, 300MHz): δ (ppm)

161.76 (1C), 154.61 (1C), 148.76 (1CH), 148.18 (1C), 145.17 (1C), 137.90 (1 CH), 127.33 (1CH), 123.59 (1C), 122.91 (1CH), 26.65(1CH2), 23.56(1CH2), 23.23 (2CH2);

3. Production of Compound 19



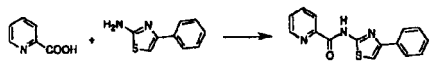
19a 19

Add dichloromethane (1 ml) to a mixture of compound 19a (28 mg, 0.197 mmol), 2- carboxyl pyridine (25 mg, 0.197 mmol) and DCC (43 mg, 0.20 mmol), DMAP (cat), and stir for 8 hours at room temperature under Ar gas protection. Dilute with ethyl acetate, filtrate, and remove the solvent by distillation, purify the residue with column spectra (petroleum ether: ethyl acetate= 5:1, V/V), and 14 mg white solid matter, product 19, is obtained. The production ratio is 75.7%.

^{1}H NMR ($CDCl_3$, 300MHz):

δ (ppm) 11.02 (s., 1H), 8.61 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.27 (d, *J* = 7.5 Hz, 1H), 7.91 (dt, *J* = 7.5, 7.5, 1.5 Hz, 1H), 7.50 (ddd, *J* = 7.5, 4.8, 1.5 Hz, 1H) , 2.62 (q, *J* = 7.5, 7.5, 7.5 Hz, 2H), 2.34 (s, 3H), 1.22 (t, *J* = 7.5, 7.5 Hz, 3H),;

4. **Production of Compound 22**



22a 22

Add dichloromethane (2 ml) to a mixture of compound 22a (23 mg, 0.131 mmol), 2-carboxyl pyridine (17 mg, 0.131 mmol, 1 eq) and DCC (29 mg, 0.14 mmol), DMAP (cat), and stir for 8 hours at room temperature under Ar gas protection. Dilute with ethyl acetate, filtrate, and remove the solvent by distillation, purify the residue with column spectra (petroleum ether: ethyl acetate= 5:1, V/V), and 21 mg cyan solid matter, product 22, is obtained.

^{1}H NMR ($CDCl_3$, 300MHz):

δ (ppm) 11.25 (s., 1H), 8.66 (d, *J* = 4.5 Hz, 1H), 8.30 (d, *J* = 7.8 Hz, 1H), 7.92 (dd, *J* = 7.8, 7.8 Hz, 1H), 7.89 (d, *J* = 7.8 Hz, 2H), 7.53 (dd, *J* = 7.8, 4.5 Hz, 1H), 7.43 (t, *J* = 7.8, 7.8 Hz, 2H), 7.33 (t, *J* = 7.8, 7.8 Hz, 1H), 7.22 (s, 1H);

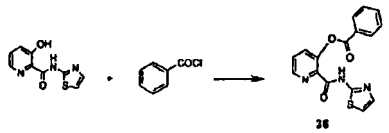
^{13}C NMR ($CDCl_3$, 300MHz): δ (ppm)

162.34 (1C), 157.45 (1C), 150.73 (1C), 148.86 (1CH), 147.98 (1C), 138.02 (1 CH),

134.63 (1C), 128.98 (2CH), 128.27 (1CH), 127.59 (1CH), 126.34 (2CH),

123.08 (1CH), 108.21 (1CH);

5. **Production of Compound 36**



36

Dissolve compound 33 (20 mg, 0.09 mmol) into dichloromethane (4 ml), add in triethylamine (0.1 ml), inject benzoyl chloride (0.13 mmol, 1.5 eq) at –78°C, react at

the same temperature for 3 hours, allow the temperature to rise naturally to room temperature and maintain the reaction for 2 hours. A light yellow solution is obtained. Dry out the residue after dehydrating with dichloromethane/toluene, purify with column spectra (petroleum ether: ethyl acetate= 3:1, V/V), and compound 36 is obtained.

^{1}H-NMR ($CDCl_3$, 300MHz):

δ (ppm) 11.33 (br., 1H), 8.60 (d, *J* = 4.5 Hz, 1H), 8.29 (d, *J* = 7.5 Hz, 2H), 7.75-7.63 (m, 3H), 7.56 (t, *J* = 4.5, 4.5 Hz, 2H), 7.50 (d, *J* = 3.0 Hz, 1H), 7.05 (d, *J* = 3.0 Hz, 1H).

^{13}C NMR ($CDCl_3$, 300MHz): δ (ppm)

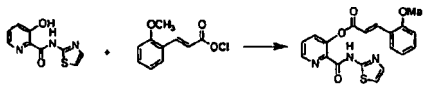
165.13 (1C), 160.29 (1C), 157.82 (1C), 148.59 (1C), 146.03 (1CH), 139.88 (1C),

137.87 (1CH), 134.12 (1CH), 133.90 (1CH), 130.85 (2CH), 129.13 (1C), 128.88 (2CH), 128.74 (1CH), 113.83 (1CH);

EIMS (m/z): 325 (M$^+$, 7%), 226(8), 197(6), 127(16), 105(69)

97(53), 91(69), 85(63), 71(100), 69(88);

7. Production of Compound 44[3]



44

Add dichloromethane (8 ml) to compound 33 (98 mg, 0.45 mmol), then add triethylamine (0.1 ml, 68 mg, 0.67 mmol), then add acyl chloride dissolved in dichloromethane at -78°C. React for 1 hour at the same temperature, then allow the temperature to rise naturally to room temperature and maintain the reaction overnight. A light yellow turbid solution is obtained. Dissolve the residue after dehydration with dichloromethane/ toluene, purify with column spectra (petroleum ether: ethyl acetate= 3:1, V/V), and product 48 mg white solid matter, which is compound 44.

^{1}H-NMR ($CDCl_3$, 300MHz):

δ (ppm) 8.55 (dd, *J* = 4.2, 1.5 Hz, 1H), 8.25 (d, *J* = 16 Hz, 1H) , 7.69-7.59 (m, 3H), 7.51 (d, *J* = 3.6 Hz, 1H) , 7.40 (dt, *J* = 8.0, 1.5 Hz, 1H), 7.03-6.94 (q, *J* = 8.0, 18 Hz, 2H), 6.99 (d, *J* = 3.6 Hz, 1H), 6.89 (d, *J* = 3.6 Hz, 1H);

^{13}C NMR ($CDCl_3$, 300MHz): δ (ppm)

165.67 (1C), 160.45 (1C), 158.89 (1C), 157.80 (1C), 148.49 (1C), 145.75 (1 CH),

143.47 (1CH), 139.98 (1CH), 138.09 (1CH), 133.86(1CH), 132.35(1CH), 129.76(1CH), 128.62 (1CH), 123.16 (1C), 120.91 (1CH), 116.93 (1CH), 113.75 (1CH),

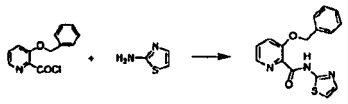
111.38 (1CH), 55.67 (1CH3),

EIMS (m/z): 381 (M$^+$, 7%), 353(8), 324(16), 225(52), 221(28)

161(90), 127(28), 123(47), 95(49), 71(60), 69(100).

8. Production of Compound 47

[3] Translator's note: numbering of original text followed. This should probably be 'Section 6'.



47

Add dichloromethane (2 ml) to acyl chloride, then add 2-aminothiazole (11.4 mg, 0.114 mmol), then add triethylamine (19 µl, 0.114 mmol) with Ar gas protection, react overnight. Add dichloromethane (10 ml) after dehydration, wash with water (3 X 3 ml), wash with saturated salt water solution (3 ml). Desiccate (with anhydrous $MgSO_4$). Filtrate, then concentrate the residue liquid, dissolve the residue into dichloromethane, purify with column spectra (petroleum ether: ethyl acetate= 1:1, V/V), 6 mg of red solid matter is produced, which is compound 47.

^{1}H-NMR ($CDCl_3$, 300MHz):

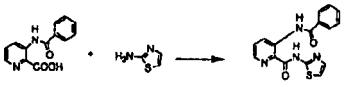
δ (ppm) 8.25 (dd, *J* = 2.7, 2.7 Hz, 1H), 7.52-7.50 (m, 3H), 7.42-7.32 (m, 5H), 7.02 (d, *J* = 3.6 Hz, 1H);

^{13}C NMR ($CDCl_3$, 300MHz): δ (ppm)

161.30 (1C), 158.38 (1C), 156.33 (1CH), 140.73 (1 CH),137.80 (1CH), 136.47 (1C), 135.85 (1C), 129.06 (2CH), 128.54 (1CH), 128.42 (1CH), 127.12 (1CH), 127.06 (1CH), 123.50 (1CH), 113.64 (1CH), 71.14 ($1CH_2$);

EIMS (m/z): 311 (M^+, 6%), 220 (7), 197 (8), 189 (43), 123 (19), 111 (29), 97 (44), 91 (100), 85 (55), 71 (83), 69 (78).

9. Production of Compound 51



51a 51

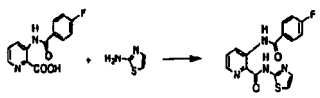
Add DDC (41 mg, 0.198 mmol), DMAP (10 mg, 0.083 mmol, 0.5 eq) and an activated 4A molecular sieve (100 mg) to 51a (40 mg, 0.165 mmol). Inject redistilled toluene (1 ml) with Ar gas protection, stir for 20 minutes at room temperature, add 2-aminothiazole (17 mg, 0.165 mmol), stir for 1.5 hours at 70°C. Filtrate, adding the filtrate directly into the upper column, and purify with column spectra (petroleum ether: ethyl acetate= 3:1, V/V). 4 mg of white solid matter is produced.

^{1}H-NMR ($CDCl_3$, 300MHz):

δ (ppm) 9.40 (dd, *J* = 8.4, 1.5 Hz, 1H), 8.36 (dd, *J* = 4.5 Hz, 1H), 8.12- 8.09 (m, 2H), 7.62-7.55 (m, 5H), 7.09 (d, *J* = 3.6 Hz, 1H);

EIMS (m/z): 324 (M^+,7), 239 (6), 225 (19), 197 (11), 125 (25), 121 (11), 111 (39), 97 (60), 85 (65), 71 (100), 69 (96).

10. Production of Compound 53



53a 53

Add DDC (77 mg, 0.375 mmol, 1.3 eq), DMAP (18 mg, 0.144 mmol, 0.5 eq) and an activated 4A molecular sieve (100 mg) to 53a (75 mg, 0.288 mmol). Inject redistilled toluene (1 ml) with Ar gas protection, stir for 20 minutes at room temperature, add 2-aminothiazole (29 mg, 0.288 mmol), stir for 3 hours at 70°C. Filtrate, adding the filtrate directly into the upper column, and purify with column spectra (petroleum ether: ethyl acetate= 1:1, V/V), 13 mg of white solid matter is produced, which is compound 53.

^{1}H-NMR ($CDCl_3$, 300MHz):

δ (ppm) 12.49 (s, 1H), 11.38 (br., 1H), 9.36 (dd, *J* = 8.4, 0.9 Hz, 1H), 8.36 (dd, *J* = 4.5, 0.9 Hz, 1H), 8.14-8.10 (m, 2H), 7.61-7.57 (m, 2H), 7.28-7.22 (m, 2H), 7.10 (d, *J* = 3.6 Hz, 1H);

^{1}H-^{1}H-COSY NMR ($CDCl_3$, 300MHz):

Correlation Peaks: CH-CH-CH, CH(2H)-CH(2H), CH-CH;

^{13}C NMR ($CDCl_3$, 300MHz): δ (ppm)

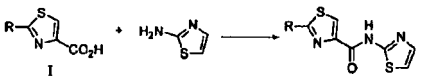
167.27 (1C), 165.53 (1C), 165.12 (1C), 163.91 (1C), 157.06 (1C), 142.79 (1CH),

139.47 (1C), 138.62 (1CH), 131.52 (1C), 130.33 (1CH), 130.21 (1CH), 129.17 (1CH), 129.04 (1CH), 116.45 (1CH), 116.16 (1CH), 114.46 (1CH);

EIMS (m/z): 324 (M^+, 6), 239 (6), 225 (19), 197 (11), 125 (25), 121 (11), 111 (39), 97 (60), 85 (65), 71 (100), 69 (96).

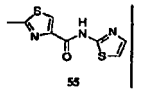
11. Production of Compound 55- 59

Compound 55- 59 are produced according to the following common formula:



I

Add 10 mL redistilled DMF into the mixture of I (1 mmol), 2-aminothiazole (120 mg, 1.2 mmol), HOBT (162 mg, 1.2 mmol), EDC (230 mg, 1.2 mmol) and small volume of 4Å molecular sieve, react overnight with rt stirring. Dilute with ethyl acetate, filtrate, and water wash 3 times to remove DMF, wash with saturated salt water solution, desiccate with organic facies anhydrous $MgSO_4$, remove the solvent by revolving distillation, and purify the residue with column spectra.

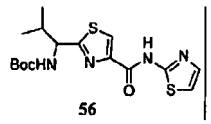


55

Compound 55: ^{1}H-NMR ($CDCl_3$, 300MHz): δ (ppm) 11.08 (br, 1H), 8.12 (s, 1H), 7.53 (d, *J* = 3.6 Hz, 1H), 7.00 (d, *J* = 3.6 Hz, 1H), 2.70 (s, 3H)

13C NMR ($CDCl_3$, 300MHz): δ (ppm)

166.87 (1C), 158.54 (1C), 158.04 (1C), 147.70 (1C), 138.30 (1CH), 125.59 (1CH), 113.79 (1CH), 19.25 (1CH3);

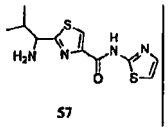


56

Compound 56: ^{1}H-NMR ($CDCl_3$, 300MHz): δ (ppm) 10.64 (br, 1H), 8.20 (s, 1H), 7.49 (d, *J* = 3.6 Hz, 1H), 7.02 (d, *J* = 3.6 Hz, 1H), 5.30 (d, *J* = 8.7 Hz, 1H), 4.89 (m, 1H), 2.32 (m, 1H), 0.97 (d, *J* = 6.9 Hz, 1H), 0.91 (d, *J* = 6.9 Hz, 1H);

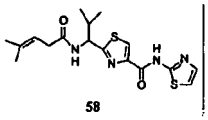
^{13}C NMR ($CDCl_3$, 300MHz): δ (ppm)

173.84 (1C), 158.31 (1C), 157.78 (1C), 155.55 (1C), 147.97 (1C), 138.10 (1CH), 125.36 (1CH), 114.14 (1CH), 80.52 (1C), 58.10 (1CH), 33.39 (1CH), 28.44 (3CH3), 19.41 (1CH3), 17.59 (1CH3);



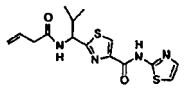
57

Compound 57: ^{1}H NMR ($CDCl_3$, 300MHz) δ (ppm): 8.66 (br, 2H), 7.63 (d, *J* = 3.9 Hz, 1H), 7.21 (d, *J* = 3.9 Hz, 1H), 4.86 (m, 1H), 2.50 (m, 1H), 1.11 (d, *J* = 6.9 Hz, 1H), 0.98 (d, *J* = 6.9 Hz, 1H);



58

Compound 58: ^{1}H NMR ($CDCl_3$, 300MHz) δ (ppm): 8.22 (s, 1H), 7.51 (d, *J* = 3.6 Hz, 1H), 7.05 (d, *J* = 3.6 Hz, 1H), 6.60 (d, *J* = 8.7 Hz, 1H), 5.38 (m, 1H), 5.24 (dd, *J* = 8.7, 5.7 Hz, 1H), 3.06(d, *J* = 7.8 Hz, 2H), 2.38 (m, 1H), 1.82 (s, 3H), 1.69 (s, 3H), 0.88 (d, *J* = 6.9 Hz, 3H), 0.86 (d, *J* = 6.9 Hz, 3H);

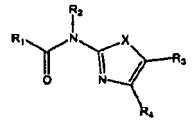


59

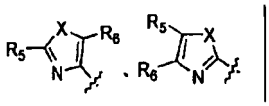
Compound 59: ^{1}H NMR ($CDCl_3$, 300MHz) δ (ppm): 8.22 (s, 1H), 7.50 (d, *J* = 3.6 Hz, 1H), 7.05 (d, *J* = 3.6 Hz, 1H), 6.77 (d, *J* = 8.7 Hz, 1H), 6.00 (m, 1H), 5.26 (m, 3H), 3.12 (d, *J* = 6.9 Hz, 2H), 2.33 (m, 1H), 0.97 (d, *J* = 6.6 Hz, 3H), 0.96 (d, *J* = 6.6 Hz, 3H)

CLAIM STATEMENT

1. **A new type of methionine aminopeptidase inhibitor with the following formula:**



Wherein R_1 represents alkyl at C1-C4, alkyl substituent, cycloalkyl at C_3-C_6, cycloalkyl substituent, aryl, pyridyl; aryl substituted by alkyl at C1-C4, nitryl, carboxyl, aldehyde group, alkoxyl, amidocyanogen, amide group, hydrosulfide group, pyridyl substituent, and heterocycle or substituent heterocycle with the following structure:



R_5, R_6 represent H, alkyl at C1-C4, alkyl substituent, cycloalkyl at C_3-C_6, cycloalkyl substituent, aryl, pyridyl, nitryl, carboxyl, aldehyde group, alkoxyl, amidocyanogen, amide group, aryl substituted by mercapto, pyridyl substituent;

R_2 represents H, alkyl at C1-C4, alkyl substituent, aryl, aryl substituted by alkyl at C1-C4, nitryl, carboxyl, aldehyde group, alkoxyl, amidocyanogen, amide group or mercapto;

R_3 represents H, alkyl at C1-C4, alkyl substituent at C1-C4, halogen; aryl, aryl substituent;

R_4 represents H, alkyl at C1-C4, alkyl substituent, aryl substituent;

X represents O, S, N, hetero atoms.

2. **Features of the methionine aminopeptidase inhibitors described in Claim 1 are:**

When R_1 is pyridine, pyridine substituents including halogen, amide, alkoxyl, hydroxyl, carboxyl, ester group, ether,

R_2 is H;

R_3 is H, Br, alkyl;

R_4 is H, alkyl, aryl substituent.

3. **Features of the methionine aminopeptidase inhibitors described in Claim 1 are:**

When R_1 is aryl, aryl substituents including nitryl, amidocyanogen, alkoxyl at C1-C4, hydroxyl, carboxyl, benzyl [Source illegible]

R_2 is H;

R_3 is H, halogen, alkyl at C1-C4,

R_4 is H, alkyl at C1-C4, aryl substituent.

4. **Features of the methionine aminopeptidase inhibitors described in Claim 1 are:**

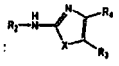
When R_1 is heterocyclic or substituent heterocyclic

R_2 is H;

R_3 is H.

R_4 is H,

R_5, R_6 is H, alkyl at C1-C4, alkyl substituent, cycloalkyl at C_3-C_6, cycloalkyl substituent, aryl, pyridyl, nitryl, carboxyl, aldehyde group, alkoxyl, amidocyanogen, amide group, aryl substituted by mercapto, pyridyl substituent;

5. A feature of the production methods of the methionine aminopeptidase inhibitors described in Claim 1 is condensation polymerisation between R_1COY (wherein Y represents hydroxyl, halogen or other active groups) and 

6. A feature of the production methods of the methionine aminopeptidase inhibitors described in Claim 4 is that the condensation agents are DCC, EDC, DIC, HBTU.

7. A feature of the production methods of the methionine aminopeptidase inhibitors described in Claim 4 is that the solvents for condensation reaction are dichloromethane, dimethylfuran, dichloroethane, toluene, benzene, water, Dioxane or mixture solvent of the above solvents.

8. A feature of the production methods of the methionine aminopeptidase inhibitors described in Claim 4 is that the reaction temperature is -20°C to room temperature or heated temperature between 50°C and 130°C.

9. A feature of the production methods of the methionine aminopeptidase inhibitors described in Claim 4 is that activators such as HOBT, pentafluorophenol, or a molecular sieve are added to the condensation reaction.

10. A feature of the production methods of the methionine aminopeptidase inhibitors described in Claim 4 is that catalysts such as triethylamine, diethyl propyl ethylamine, pyridine, DMAP alkali are employed in the condensation reaction.

11. The methionine aminopeptidase inhibitors described in Claim 1 are used as leading compounds in anti-tumour or anti-infection drugs.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN03/00213

A. CLASSIFICATION OF SUBJECT MATTER

C07D277/22 277/38 263/30 263/48 207/30 207/34 401/12 403/12 405/12 407/12 417/12 A61K31/40 31/41

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99/57098 A (11 Nov.1999, the whole doc.)	1-11
X	CN 1033626 A(5 July 1999, the whole doc.)	1-11
X	CN 1035826 A (27 Sep. 1989, the whole doc.)	1-11

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim (S) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document refering to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
June 16, 2003	24 JUL 2003 (24.07.03)
Name and mailing address of the ISA/CN 6 Xitucheng Rd., Jimen Bridge, Haidian District, 100088 Beijing, China Facsimile No. 86-10-62019451	Authorized officer Zhou Hub Telephone No. 86-10-62093075

Form PCT/ISA /210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN03/00213

Box I Observations where certain claims were found unsearch able (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos: 1(part)
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

In claim 1 the terms such as "substituted aryl" define too broad a scope that it is impossible for the examiners to do a completed search, so the search is mainly based on the related embodiment of description (in which X=S).

3. ☐ Claims Nos:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant,this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant.Consequently ,this international search report is restricted to the invention first mentioned in the claims;it is covered by claims Nos.:

Remark on protest ☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (cotinuation of first sheet (1)) (July 1998)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN03/00213

WO 99 57098 A	19991111		
		AU3877899 A	19991123
		NO200005506 A	20001229
		EP1073633 A2	20010207
		CZ200003967 A3	20010314
		SK200001628 A3	20010510
		KR2001043198 A	20010525
		CN1308608 A	20010815
CN 1033626 A	19890705	EP0296673 A	19881228
		AU1832988 A	19890105
		JP1026572 A	19890127
		DK8803467 A	19881226
		US4877802 A	19891031
		SU1579458 A	19900715
		EP0296673 B1	19940309
		DE3888245G G	19940414
		CA1328867 C	19940426
		ES2061628T T3	19941216
		DK169743B B	19950213
CN 1035826 A	19890927	EP0313091 A	19890426
		AU2414788 A	19890427
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		HU49595 A	19891030
		JP2045402 A	1990021
		JP2067207 A	19900307
		JP2149572 A	19900608
		US4980363 A	19901225
		DD283323 A	19901010
		KR9006746 B	19900920
		US5104886 A	19920414
		DD296401 A	19911205
		DD296484 A	19911205
		DD296490 A	19911205
		RO102459 A	19911030
		CN1066363 A	19921125
		EP0313091 B1	19930901
		DE3883695G G	19931007
		RO106645 B1	19930630
		RO107181 B1	19931029
		RO107182 B1	19931029
		ES2058209T T3	19941101
		RO107337 B1	19931130
		RO107338 B1	19931130
		RO107654 B1	19931230
		RO107655 B1	19931230
		JP2648621B2 B2	19970903
		JP2723155B2 B2	19980309
		JP2788458B2 B2	19980820

Translation from Chinese

(Page 21, 22 and 23)

(Chinese Contents are mainly the same as the English Contents in Page 18, 19 and 20, except for in the <INTERNATIONAL SEARCH REPORT>, the date in the (left) 12th box from the top and the item in the 10th box from the top, which is selected, as compared to those in Page 19.)
